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SPECIFICITY OF AN INTERFERENCE AMONG GROUP A ARBOVIRUSES

Matteo A. Cardella
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DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland

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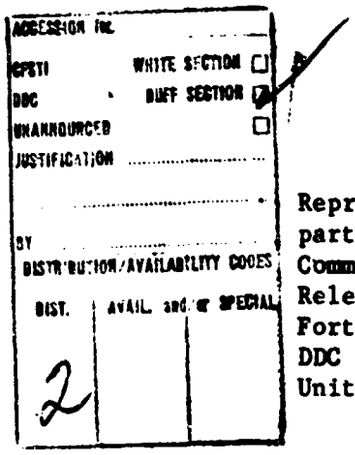

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DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland 21701

TECHNICAL MANUSCRIPT 535

SPECIFICITY OF AN INTERFERENCE AMONG GROUP A ARBOVIRUSES

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Project 1B061102B71A

June 1969

ABSTRACT

Studies on a non-interferon-mediated type of interference among the group A arboviruses, eastern equine encephalitis (EEE) virus, and a temperature-sensitive mutant (Ets-4) of EEE, have been extended to include Newcastle disease virus (NDV), vesicular stomatitis virus (VSV), and rabbit pox (RP) virus.

In the presence of actinomycin D, Ets-4 virus interfered with the growth of VSV and NDV. The interference observed between NDV or VSV and Ets-4 was reciprocal. VSV did not deter the growth of NDV, but appeared to stimulate its growth slightly. Interference of the growth of VSV by NDV was not observed. The lack of interference between NDV and VSV was therefore shown to be reciprocal.

Interference between EEE and RP viruses was not observed when either virus was used as the first infecting virus. EEE virus, however, stimulated the growth of RP virus in tests for interference effects. The stimulatory effect was increased by increasing the MOI of EEE virus or decreasing that of the RP virus.

The results summarized above demonstrate that interference among group A arboviruses extends to NDV and VSV. With the Ets-4 virus this interference is reciprocal. The lack of interference between NDV and VSV in these experiments suggests that a member of the group A arboviruses is required for the type of interference observed, although the failure to demonstrate interference between EEE and RP viruses presents a contradiction. The stimulatory effect of EEE virus on RP virus early in the infection cycle suggests that both events may represent an early activity manifested by the viral genomes. The mechanism of interference and extent of the observed stimulation remain to be elucidated.

I. INTRODUCTION*

Interference of viral growth mediated by interferon has now been firmly established. The role of the interfering virus in this type of interference is that of an inducer of interferon. Several other types of viral interference have been reported that did not involve mediation by interferon. Among these was the interference described by Cords and Holland among different strains of poliovirus.** Replication of the interfering virus for a variable length of time was necessary before cells became resistant to superinfection with challenge virus. Cords and Holland suggested that this type of interference might be due to direct competition for substrates or replication sites within the host cells. A similar type of interference has recently been reported among the group A arboviruses by Zebovitz and Brown.*** Interference was observed only if the interfering virus had a growth advantage over the challenge virus either in time or in multiplicity of infection. Preliminary experiments were also reported that indicated that this type of interference extended to and included both the Newcastle disease virus and the vesicular stomatitis virus, and that this interference was reciprocal.

This report confirms that this type of interference can be extended to include both the Newcastle disease and vesicular stomatitis viruses. In addition, the stimulation of the rabbit pox virus by a group A arbovirus under conditions of interference is also reported.

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* This report should not be used as a literature citation in material to be published in the open literature. Readers interested in referencing the information contained herein should contact the senior author to ascertain when and where it may appear in citable form.

** Cords, C.E.; Holland, J.J. 1964. Interference between enteroviruses and conditions effecting its reversal. Virology 22:226-234.

*** Zebovitz, E.; Brown, A. 1968. Interference among group A arboviruses. J. Virol. 2:1283-1289.

II. MATERIALS AND METHODS

In the experiments reported here the viruses used to induce interference or used as challenge virus were eastern equine encephalitis (EEE) virus; a temperature-sensitive mutant of EEE virus, the Ets-4; NDV (Baron); VSV (N.J.); and the rabbit pox virus (U.C. strain).

Interference in the growth of the challenge virus was examined by inoculating chick monolayer cells with the "interfering" virus 3 to 4 hours before superinfecting the cultures with the challenge virus. Multiplicities of 10:1 were employed throughout, except in those experiments involving EEE and RP viruses. A 30-min pretreatment with 1 μg of actinomycin D per ml, as well as inclusion in the liquid overlay medium, was used to limit induction of interferon. Actinomycin D was not used in the experiments involving the rabbit pox virus. Samples were taken at intervals over a 24-hour period; the time when cultures were infected with super-infecting virus was considered zero time.

III. RESULTS

Interference in the growth of challenge virus, VSV or NDV, was demonstrated, using the Ets-4 as the interfering virus.

Figure 1 shows the interference of VSV that resulted when Ets-4 virus was inoculated onto cells 4 hours before the VSV. The VSV titers in the doubly infected cultures were more than two logs below those of the VSV control cultures, and the interference reached a maximum 4 to 6 hours after the cultures were superinfected with the VSV virus. A decline in the interference to approximately one log was noted later in the incubation period.

Figure 2 shows the interference of NDV growth that resulted when Ets-4 was inoculated onto cells 4 hours before NDV. Maximum interference was observed 8 to 9 hours after the cultures were superinfected with NDV, then declined slightly but at 24 hours equaled that observed earlier. The interference observed was slightly less than that observed with VSV. These results are similar to those observed by Zebovitz and Brown* among the group A arboviruses, and suggest that the interference might involve some early stage in the infectious cycle of challenge virus.

Reciprocal interference among group A arboviruses was previously demonstrated by Zebovitz and Brown.* Consideration was therefore given to determining if a reciprocal interference relationship existed between NDV or VSV and the Ets-4 viruses.

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* Zebovitz, E.; Brown, A. 1968. Interference among group A arboviruses. J. Virol. 2:1283-1289.

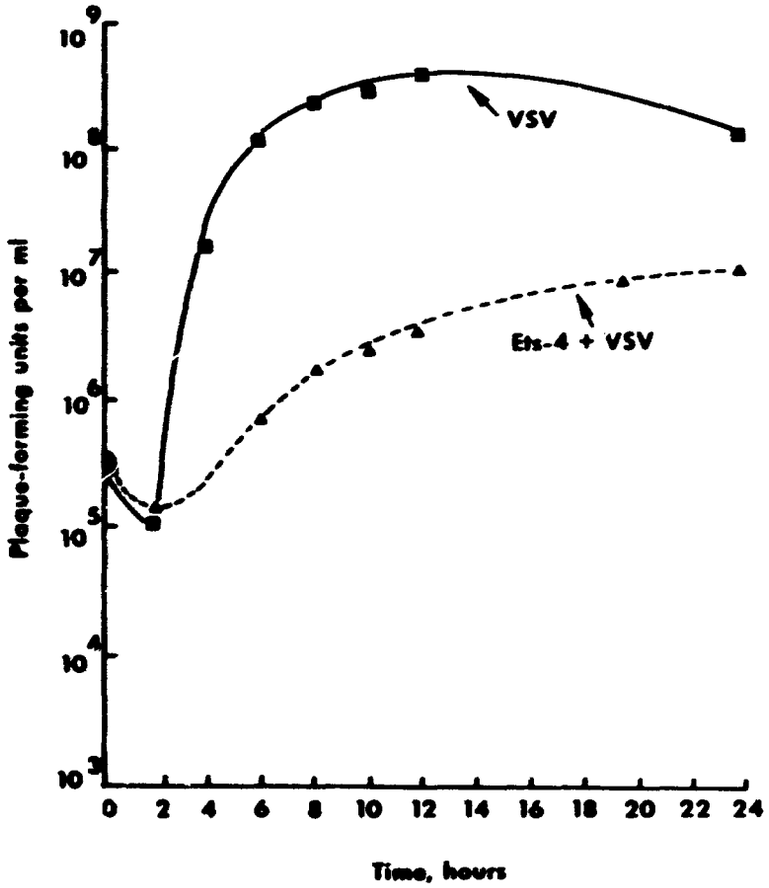

FIGURE 1. Interference of VSV Growth by Ets-4.

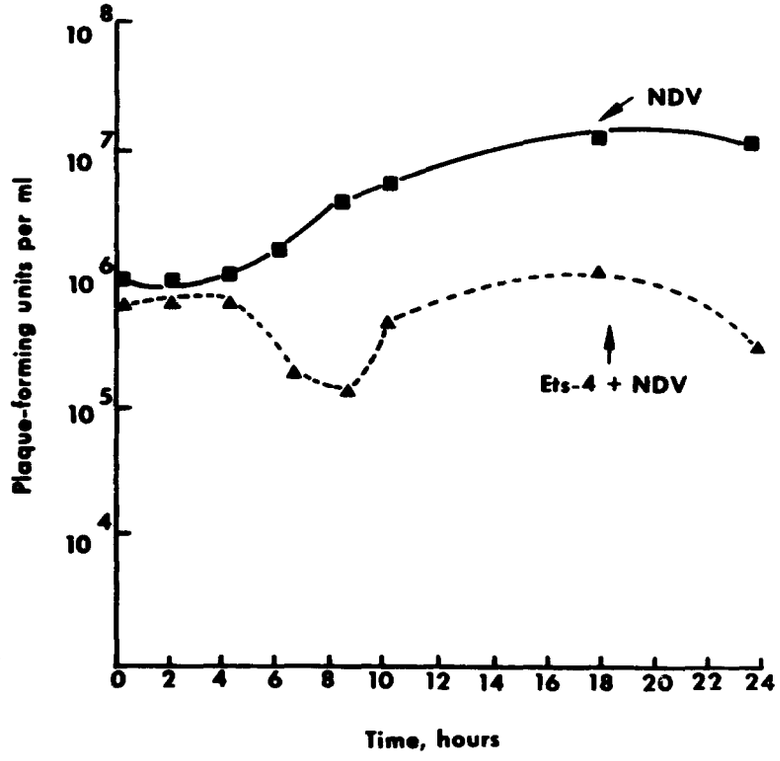

FIGURE 2. Interference of NDV Growth by Ets-4.

Figure 3 shows the results obtaine ' when VSV was used as the interfering virus 4 hours before superinfection with Ets-4 virus. Interference in the growth of Ets-4 virus was observed at 9 hours after Ets-4 challenge and increased to more than two logs at 24 hours. Essentially similar results were obtained when NDV was used as the interfering virus, although the interference was more pronounced earlier in the incubation period.

Figure 4 shows the results obtained when NDV was inoculated onto cells 4 hours before the Ets-4 virus. Ets-4 titers in doubly infected cultures were approximately 1.6 logs lower than those of control cultures. These data confirm the earlier observations of Zebovitz and Brown, and show that interference observed among group A arboviruses extends to the Newcastle disease and vesicular stomatitis viruses and that it is reciprocal.

In contrast to these findings, VSV did not interfere with the NDV, nor did NDV interfere with the VSV, when either one was used as interfering virus. The lack of interference between these viruses appears to be reciprocal.

A summary of the breadth of the type of interference described is shown in Table 1. The degree of interference induced by Ets-4 ranged from 1.8 to 2.2 logs when NDV or VSV ($\leftrightarrow$) was used as challenge virus. The reciprocal nature of the interference is shown by the fact that either VSV or NDV ($\updownarrow$) interfered with the Ets-4 virus. Reciprocal lack of interference was observed between the vesicular stomatitis and Newcastle disease viruses.

Studies on the observed interference were extended to include a DNA virus, the rabbit pox virus. No evidence was obtained to indicate that EEE virus interfered with the growth of the rabbit pox virus. In addition, the RP virus was not shown to interfere with the growth of EEE virus. However, it was noted that, although EEE virus did not deter the growth of the RP virus, it appeared to exert a stimulatory effect.

Figure 5 shows the stimulation of RP virus growth that resulted when EEE virus was inoculated onto cells 4 hours before superinfection with the RP virus. The RP virus titers in the doubly infected cultures were 1.0 to 1.8 logs above those observed for the control cultures early in the incubation period.

The observed stimulation could also be demonstrated and further increased by decreasing the RP virus multiplicity of infection (MOI).

A more marked stimulation was observed when the MOI of the EEE virus was increased to 100 and the RP virus was inoculated at a multiplicity of infection of 1.0. Figure 6 shows that under these conditions RP virus titers in the doubly infected cultures were approximately 3.5 logs higher than those obtained in control cultures at 2 to 4 hours after superinfection with the RP virus. It appears that the stimulatory effect is manifest at an early stage in the replication of the superinfecting RP virus. The extent and nature of the observed stimulation remain to be elucidated.

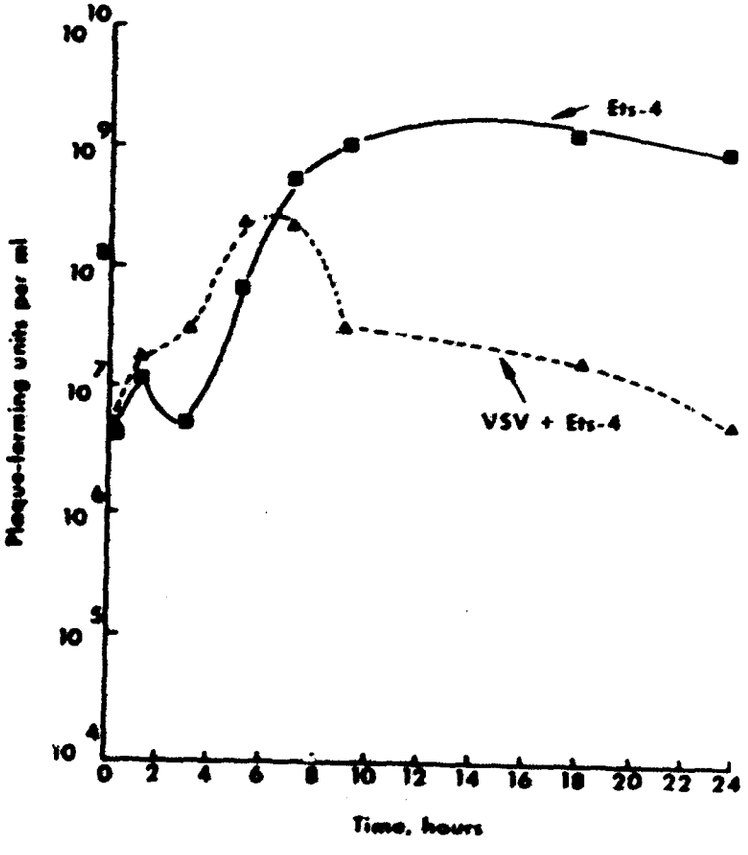

FIGURE 3. Interferance of Ets-4 Growth by VSV.

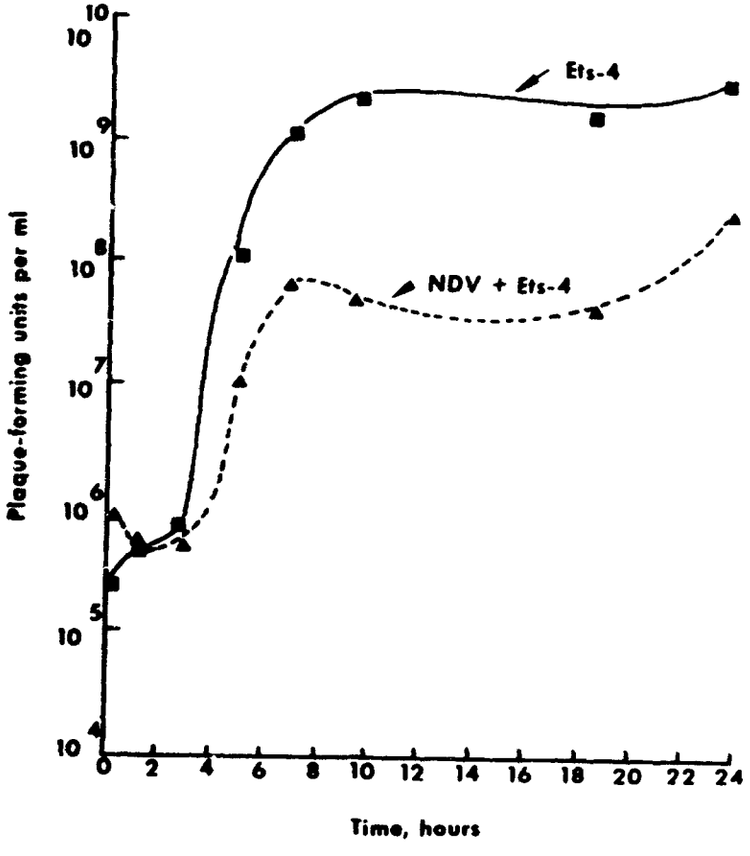

FIGURE 4. Interference of Ets-4 Growth by NDV.

TABLE 1. DEGREE OF INTERFERENCE IN LOG_{10} UNITS

Interfering Virus	Challenge Virus		
	Ets-4	VSV	NDV
Ets-4	-	2.2	1.8
VSV	2.3	-	0.0
NDV	1.6	0.0	-

IV. SUMMARY

In summary, the non-interferon-mediated interference previously observed among group A arboviruses has been shown to extend to and include both the vesicular stomatitis and Newcastle disease viruses and to be reciprocal. No interference was demonstrated between VSV and NDV. Interference between EEE and RP viruses was not observed when either virus was used as the first infecting virus. EEE virus was shown, however, to stimulate the growth of RP virus in tests for interference effects. The stimulatory effect was increased by increasing the MOI of EEE virus and/or decreasing that of the RP virus.

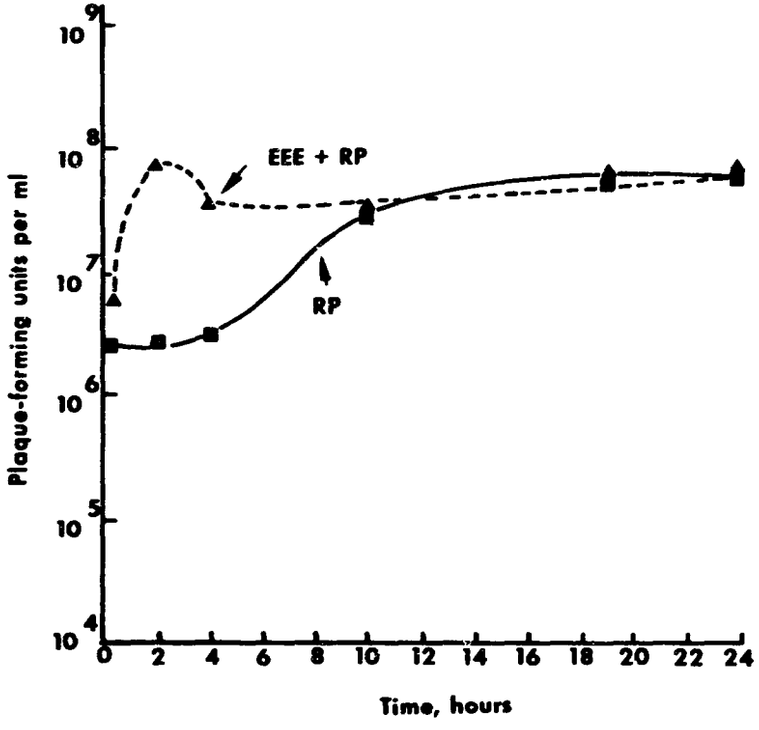

FIGURE 5. Stimulation of RP Virus Growth by EEE Virus.

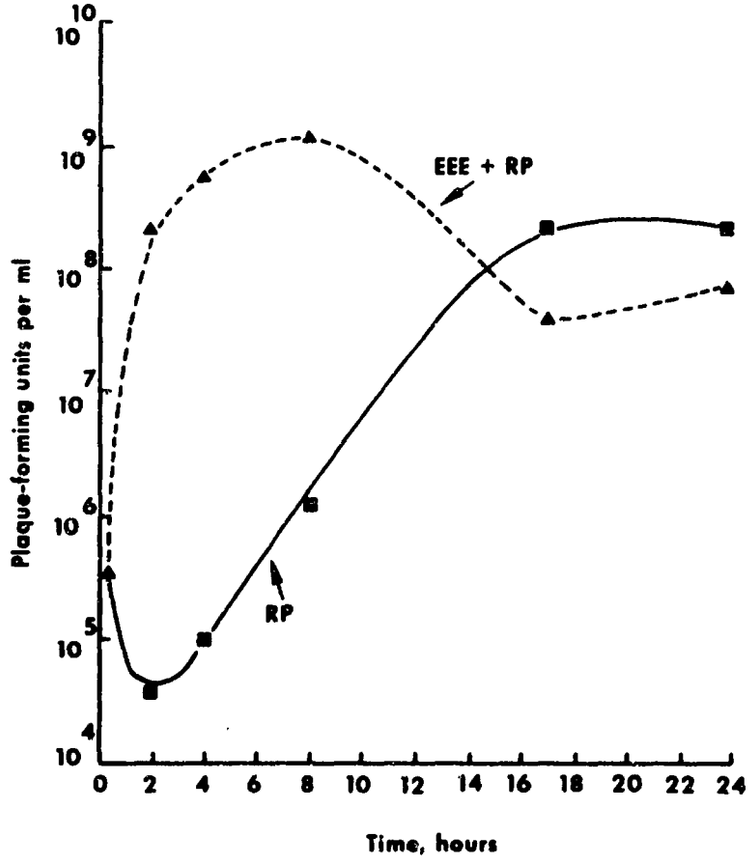

FIGURE 6. Stimulation of RP virus Growth by EEE Virus: Effect of MOI.

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14. KEY WORDS	LINK A		LINK B		LINK C	
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* Arboviruses						
* Group A arboviruses						
* Viral interference						
Eastern equine encephalitis virus						
Viruses						
Chick embryo						

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